STIC-ILL

NQS QH604.C442

From: Schmidt, Mary
Sent: Thursday, December 12, 2002 2:48 PM
To: STIC-ILL
Subject: last set of references 09/122,588

AU 1635

424059

VEGF references:

Thierry et al., Proceedings of the american association for cancer research annual meeting, 36, 0, p413, 1995.

Yung et al., neurology 48, 3 suppl., 2, pa22, 1997.

ke et al., proceedings of the american association for cancer research annual meeting, 37, 0, p60, 1996.

ellis eta l., surgery, nov. 1996, 120, 5, p871-8.

martiny-baron et al., current opinion in biotechnology, dec. 1995, 6, 6, p675-80.

warren et al., journal of clinical investigation, apr. 1995, 95, 4, p1789-97.

o-brien et al., cancer research , feb. 1, 1995, 55, 3, p510-3.

chaudhary et al., molecular biology of the cell, 7, suppl., p352a, 1996.

saleh et al, cancer research, 1-15-1996, 56, 2, 393-401.

claffey et al, cancer research, 1-1-1996, 56, 1, 172-81.

levy et al, j. of biological chem, 10-11-1996, 271, 41, p25492-7.

thanks,
Melissa
11e12 mailboxes

(2)
.40¢ L.C
12/13

Color separation can take generations. . . with POROS® media, separations take minutes.

The unique structure of POROS media enables liquid chromatography of biomolecules to be performed 10 to 100 times faster than in comparison to traditional media, with no loss of resolution or capacity.

Patented POROS media contain channels that allow liquid to flow directly into the column packing beads. Inside the beads, the channels carry biomolecules to a vast network of accessible binding sites—enabling the use of high flow rates while maintaining superior separation.

Optimize all variables for maximum yield and purity

- Improve yield of active biomolecules by limiting the time spent under potentially denaturing conditions
- Reduce costs by using smaller columns that can be cycled rapidly
- Gain time to screen different modes of chromatography and to optimize separation conditions

Flexibility and compatibility to meet your specific needs

Compatibility	All conventional low (peristaltic pumps), medium (FPLC®), and high pressure (HPLC) chromatography systems
Modes of Chromatography Available	Ion exhange, hydrophobic interaction (HIC), metal chelate, affinity, activated affinity, reverse phase
Biomolecule Type	Proteins, peptides, nucleic acids
Scale of Purification	Analytical, semi-preparative, preparative
Column Life	Greater than 1000 uses (depending upon sample loads and column care)
Column Materials and Fittings	PEEK or stainless steel columns are HPLC-ready and supplied with adaptors for FPLC® or peristaltic pumps

Call us today and start seeing why POROS media is generations ahead of the rest.

INTRODUCING Boehringer Mannheim POROS® M A

mannheim boehringer Biochemicals

Internet http://biochem.boehringer-mannheim.com

Australia (02) 899 7999; Austria (0222) 277 87; Belgium (02) 247 4930; Brazil 55 11 66 3565; Canada (514) 686 7050; (800) 361 2070; Chile 00 56 (2) 22 33 737; China 86 21 6416 4320; Czech Republic (0324) 45 54, 58 71-2; Denmark 49 13 82 32; Finland (90) 429 2342; France 76 76 30 86; Germany (0621) 759 8545; Greece (01) 67 40 238; Hong Kong (852) 2485 7596; India (22) 837 0794; Indonesia 62 (21) 520 2820; Israel 972-3-6 49 31 11; Italy 02 270 96209; Japan 03 3432 3155; Malaysia 60 (03) 755 5039; Mexico (5) 227 8967, -61; Netherlands (036) 539 4911; New Zealand (09) 276 4157; Norway 22 07 65 00; Poland (22) 36 06 77-87; Portugal (01) 417 17 17; Republic of Ireland 1 800 409041; Russia (49) 621 759 8636; Singapore/Philippines 65 272 7500; South Africa (011) 886 2400; South Eastern Europe +43 (1) 277 87; South Korea 02 569 6902; Spain (93) 201 4411; Sweden (08) 404 88 00; Switzerland 0 41/7 99 6161; Taiwan (02) 736 7125; Thailand 66 (2) 274 0709-13; Turkey 1 349 81 76-79; United Kingdom (0800) 521 578; USA (800) 428 5433.

POROS® is a registered trademark of PerSeptive Biosystems, Inc.

FPLC® is a registered trademark of Pharmacia Biotech AB.

2041

INSULIN-LIKE GROWTH FACTOR I (IGF-I) AND ESTROGEN RECEPTORS IN NITROSOMETHYLUREA INDUCED MAMMARY TUMOURS. ((Karen S. Whittal, Silia Chadan and Wade S. Parkhouse.)) School of Kinesiology, Simon Fraser University

Insulin-like growth factor -I (IGF-I) and estrogen receptors were measured in nitrosomethylurea (NMU) induced rat mammary tumours by western blot analysis. A novel approach used in this study was the assessment of estrogen receptor by western blots as apposed to binding assays. As well, IGF-I has not previously been studied in NMU induced tumors. Antibodies directed towards the IGF-I receptor alpha and beta subunits revealed a protein band at approximately 130 kDa and 97 kDA respectively in both the normal tissue and mammary tumor. IGF-I receptor protein amount was significantly increased (33 percent) in the tumor samples with concomitant enhancement of IGF-I binding as demonstrated with binding curves. There was a weak negative correlation between tumor growth rate and IGF-I receptor content. Tumors with a final tumor weight less than three grams contained significantly more IGF-I receptors than tumors greater than three grams. Estrogen receptor content was significantly increased in the tumors (220 percent). In contrast to normal tissue, the tumors demonstrated three protein bands suggesting the presence of three isoforms of the estrogen receptor. There was no relationship between estrogen receptor content and tumor growth rate or final tumor weight. These results suggest IGF-I and estrogen receptors play a role in NMU induced mammary tumorigenesis.

2042

POTENT ANTISENSE INHIBITION OF VEGF EXPRESSION USING CHEMICALLY REDESIGNED OLIGONUCLEOTIDES. ((N. Chaudhary, Z.-Y. Li, D.A. Walker, H. Vu, D. M. Mulvey, J. O. Ojwang, T. S. Rao, G. R. Revankar, P. A. Cossum, A. Peyman*, E. Uhlmann* and R. F. Rando)). Aronex Pharmaceuticals, Inc., The Woodlands, TX, 77380, and *Hoechst A.G., Central Pharma Research, Frankfurt, Germany.

Vascular endothelial growth factor (VEGF) comprises a family of homodimeric glycoproteins (34-46 kD) necessary for blood vessel formation during normal growth and development, and tissue repair. Aberrant expression of VEGF is associated with diseases characterized by a high degree of vascularization, including aggressively growing cancers, psoriasis, rheumatoid arthritis, and diabetic retinopathy. We have focused on the discovery of antisense oligonucleotides that can inhibit VEGF expression by producer cells, and in turn, help control the pathologic vasculogenesis and angiogenesis. An ELISA-based screening procedure was used to evaluate the effect of antisense oligonucleotides on VEGF expression by human keratinocytes maintained in culture. To improve the internalization of oligonucleotides by cells, formulations were prepared using novel polyaminolipid uptake enhancers that are designed to be nontoxic and biodegradable. To increase the binding affinity of the antisense oligonucleotide for its mRNA target, the cytosines and thymidines were replaced with their C5-propynyl-containing homologues, modifications that markedly increase the T_m of duplex formation. The use of phosphorothioate internucleotide linkages provided protection from nucleases. Finally, to further increase the biological efficacy of the oligonucleotide, the 3' terminus was attached to a novel tether, with the aim of exploiting the delivery and active transport machinery to improve targeting to the cell and enhance penetration across the endosomal membrane. By screening a series of modified oligonucleotides (of varying lengths), we identified a 19-base oligomer that can specifically reduce cellular VEGF expression in the nanomolar concentration range. The potent, sequence-specific activity of the oligonucleotide combined with low toxicity suggests that formulations of this antisense oligomer may be attractive candidates for development as anti-VEGF therapeutics.

2043

PERIVASCULAR EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN OVINE CORPORA LUTEA THROUGHOUT THE ESTROUS CYCLE. ((V. Doraiswamy[1], L.P. Reynolds[2], S.D. Killilea[2], RM Moor[3], and D.A. Redmer[1])) Depts. of [1]Animal and Range Sciences and [2]Biochemistry, North Dakota State University, Fargo, ND 58105, USA, and [3]Dept. of Development and Signalling, The Babraham Institute, Cambridge, United Kingdom.

We have shown that the pattern of expression of VEGF by ovine corpora lutea corresponds to the pattern of luteal vascularization; i.e., it is greatest soon after ovulation and decreases throughout the estrous cycle (Redmer et al., J.Reprod. Fertil., 1996, In press). To immunolocalize VEGF, and thereby identify luteal cell types that produce it, CL were obtained from superovulated ewes slaughtered on Days 2, 4, 10, and 15 (n=7-9/group) of the estrous cycle, fixed in Carnoy's solution, and embedded in paraffin. VEGF was immunolocalized in luteal tissue sections (6 μm) by using a specific affinity-purified antibody (Red-1, made against a synthetic 15 amino acid N-terminal peptide of ovine VEGF) and indirect immunoperoxidase detection. Lectin BS-1 or 3β-hydroxysteroid dehydrogenase (3βHSD) also were immunolocalized along with VEGF in tissue sections to determine the identity of endothelial and steroidogenic cells, respectively. For all tissue sections, VEGF staining was primarily cytoplasmic and was exclusively in non-steroidogenic cells. VEGF was localized primarily in perivascular cells (pericytes) and arteriolar smooth muscle cells throughout the estrous cycle. On Days 2 and 4, VEGF was most prominent in cells near endothelial cells within the granulosa-derived parenchymal lobules of the newly developing CL; intense staining also was observed in the theca-derived connective tissue tracts and most of the large blood vessels. By Day 15, the CL exhibited intense VEGF staining of the entire capsule and perivascular areas of most large blood vessels, but little staining was observed elsewhere. These data demonstrate that VEGF protein is most prominent in developing ovine CL and is localized primarily in pericytes, indicating that VEGF plays an important role in luteal angiogenesis. *Grants NRI-CGP/USDA 93-37208-9278 and 93-37203-9271.*

2044

SYNTHESIS, STORAGE AND RELEASE OF THE VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF/VPF) BY HUMAN MAST CELLS. ((A. Grützkau, S. Krüger-Krasagakes, H. Kögel, C. Schwarz, B.M. Henz and A. Möller)) *Department of Dermatology, Virchow Klinikum, Humboldt Universität zu Berlin, 13353 Berlin, Germany*

Mast cells have been implicated in the generation of an angiogenic response in various in vitro- and in vivo-systems. Moreover, in several chronic cutaneous disorders that are accompanied by neovascularization a significant increase in the number of mast cells was ascertained. These findings and the capability of mast cells for intracellular storage of cytokines and growth factors led us to investigate the expression of VEGF in the human mast cell line-1 (HMC-1) and in human skin mast cells.

RT-PCR was used to examine the induction of VEGF transcripts in HMC-1 cells. Unstimulated cells constitutively expressed three splicing varients of VEGF mRNA (VEGF-121, VEGF-165 and VEGF-189). In cells stimulated by a combination of PMA and the calciumionophore A23187 an additional transcript, representing the mRNA for VEGF-206, was detectable.

At the protein level VEGF isoforms were identified in the supernatant of HMC-1 cultures by westernblotting and a VEGF-specific ELISA. During 24h of culture stimulated cells released twice as much VEGF in comparison to unstimulated cells. The spontaneous and the stimulus-induced secretion of VEGF was completely blocked in the presence of cycloheximide indicating an active releasing mechanism.

Mast cells not only synthesize and secrete VEGF, but also store this growth factor in their cell specific granules. This could be shown by flow cytometry and by post-embedding immunoelectron microscopy in mast cells isolated from human skin.

These results demonstrate VEGF as a new mast cell-associated growth factor that will enable mast cells to participate in angiogenic processes during various physiologic and pathophysiologic conditions.

2045

AUTOCRINE REGULATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION BY FIBROBLAST GROWTH FACTOR-2 (FGF-2). ((G. Seghezzi*, S. Patel*, G. Pintucci+ , A. Galloway*, D. Rifkin+, P. Mignatti*+.)) Departments of Surgery* and Cell Biology+, NYU Medical Center, New York, NY 10016.

We studied the role of FGF-2 in the control of VEGF expression in endothelial cells. Addition of recombinant FGF-2 to endothelial cells resulted in a 5-fold increase in VEGF mRNA levels. By immunoblotting with anti-VEGF antibody rFGF-2 increased the expression of VEGF165 over 3-fold but had no effect on other VEGF isoforms. Treatment with human SK-Hep1 hepatoma cell-conditioned medium, which contains no FGF-2, resulted in increased FGF-2 and VEGF mRNA levels. Anti-FGF-2 IgG blocked VEGF mRNA expression, indicating that FGF-2 modulates endothelial cell expression of VEGF in an autocrine manner. To characterize the effect of exogenous versus endo genous FGF-2 we used NIH 3T3 cells transfected with the cDNAs for either low-MrFGF-2 (18 kD FGF-2), high-Mr FGF-2 (HM FGF-2), or all FGF-2 isoforms (wt FGF-2). All FGF-2 transfectants and control cells expressed comparable levels of VEGF189. Control cells transfected with the vector alone expressed no detectable VEGF 165; in contrast, all FGF-2 transfectants had high VEGF 165 levels. The highest levels of VEGF165 were expressed by wtFGF-2 transfectants; HMFGF-2-expressing cells had VEGF165 higher than 18 kD FGF-2-expressing cells. In contrast, addition of recombinant FGF-2 to control NIH 3T3 cells had a modest effect on VEGF165 levels. Thus, exogenous and endogenous FGF-2 selectively upregulate VEGF 165 levels; endogenous FGF-2 appears to modulate VEGF165 expression in an autocrine manner and to be a more efficient effector that exogenous FGF-2.

* First and second author contributed equally.

2046

TIE2 RECEPTOR EXPRESSION AND PHOSPHORYLATION IN CULTURED CELLS AND MOUSE TISSUES. ((Thomas I. Koblizek[a], Andrew S. Runting[b], Steven A. Stacker[b], Andrew F. Wilks[b], Werner Risau[a], Urban Deutsch[a])) a Department of Molecular Cell Biology, Max-Planck-Institut für klinische und physiologische Forschung, Parkstr. 1, 61231 Bad Nauheim, Germany b Ludwig Institute for Cancer Research, P.O. Box 2008, Royal Melbourne Hospital, Victoria 3050, Australia

Accumulating experimental evidence indicates that endothelial cell growth and blood vessel morphogenesis are processes that are governed by the activity of specifically expressed receptor tyrosine kinases (RTKs). We have used two new rat monoclonal antibodies (MAbs) to study the expression and phosphorylation of one such receptor, mouse Tie2 (mTie2), in transfected cells, endothelioma cell lines and mouse tissues. The Tie2 receptor was found to be constitutively autophosphorylated when over-expressed in COS7 cells. In contrast, the endogenous Tie2 protein was not phosphorylated in endothelioma cell lines. However, in these cell lines Tie2 could be induced to become tyrosine phosphorylated, and this activation was found to be independent of Tie1. In addition, Tie2 receptor activity was also found during ongoing angiogenesis in the early postnatal mouse brain.